

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of : **Confirmation No. 4315**  
Jan BARNIKOW et al. : Attorney Docket No. 2006\_1322A  
Serial No. 10/591,159 : Group Art Unit 1751  
Filed October 3, 2006 : Examiner Yong D. Pak  
MUTANTS OF O6-ALKYLGUANINE-DNA  
ALKYLTRANSFERASE : **Mail Stop: AMENDMENT**

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**SUPPLEMENTAL RESPONSE TO RESTRICTION REQUIREMENT**

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

In response to the Office Communication dated January 21, 2009, and in further response to the Restriction/Species Election Requirement dated October 7, 2008, Applicants elect the sequence in Example 8, Table 1, second sequence. Applicants note that the elected species having the following mutations vs. human AGT:

Lys32Ile, Leu33Phe, Cys62Ala, Gln115Ser, Gln116His, Lys125Ala, Ala127Thr, Arg128Ala, Gly131Lys, Gly132Thr, Met134Leu, Arg135Ser, Cys150Gly, Ser151Gly, Ser152Asp, Ala154Asp, Asn157Gly, Ser159Glu, truncation at 182.

(Underlining means double mutations resulting in the underlined mutation)

has the following properties:

- (a) no detectable DNA interaction;
- (b) localization of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus;
- (c) at least 10-fold improved expression yield as soluble protein and improved stability in *E. coli*;

- (d) improved stability under oxidizing conditions;
- (e) at least six-fold improved stability within cells after reaction with a substrate;
- (f) improved stability outside cells before and after reaction with a substrate;
- (g) at least four-fold increased *in vitro* solubility;
- (h) 45-fold higher reactivity against O<sup>6</sup>-alkylguanine substrates;
- (i) no detectable reactivity against DNA-based substrates; and
- (j) at least 10-fold reduced reactivity against N<sup>9</sup>-substituted O<sup>6</sup>-alkylguanine substrates.

The elected species reads on claims 1-7, 9-13, 16, 17, 19, 20, 24 and 37.

In combination with the Response to Restriction/Species Election Requirement dated November 3, 2008, Applicants believe that a full response to the Restriction/Species Election Requirement dated October 7, 2008 has now been provided.

Favorable action on the merits is now requested.

Respectfully submitted,

Jan BARNIKOW et al.

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